Practitioner's Docket No. 101195-2

CHAPTER II

Preliminary Classification:

Proposed Class:

Subclass:

NOTE: *"All applicants are requested to include a preliminary classification on newly filed patent applications. The preliminary classification, preferably class and subclass designations, should be identified in the upper right-hand corner of the letter of transmittal accompanying the application papers, for example 'Proposed Class 2, subclass 129.'" M.P.E.P., § 601, 7th ed.*

TRANSMITTAL LETTER TO THE UNITED STATES ELECTED OFFICE (EO/US)

(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

PCT/DE98/03818	December 30, 1998	December 30, 1997
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED

Novel Sequence Variants of the Human Beta 2- Adrenergic Gene
TITLE OF INVENTION

Hoehe, Margaret; Timmermann, Bernd; Koepke, Karla
APPLICANT(S)

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231

ATTENTION: EO/US

CERTIFICATION UNDER 37 C.F.R. § 1.10*
(Express Mail label number is mandatory.)
(Express Mail certification is optional.)

I hereby certify that this Transmittal Letter and the papers indicated as being transmitted therewith is being deposited with the United States Postal Service on this date June 29, 2000, in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EL643059849US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Barbara LaRocca
(type or print name of person mailing paper)

Barbara LaRocca
Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. § 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

****WARNING:*** *Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. § 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

NOTE: To avoid abandonment of the application, the applicant shall furnish to the USPTO, not later than 20 months from the priority date: (1) a copy of the international application, unless it has been previously communicated by the International Bureau or unless it was originally filed in the USPTO; and (2) the basic national fee (see 37 C.F.R. § 1.492(a)). The 30-month time limit may not be extended. 37 C.F.R. § 1.495.

WARNING: *Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. § 1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing—See 37 C.F.R. § 1.8.*

NOTE: Documents and fees must be clearly identified as a submission to enter the national state under 35 U.S.C. § 371 otherwise the submission will be considered as being made under 35 U.S.C. § 111. 37 C.F.R. § 1.494(f).

I. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. § 371:

a. ☒ This express request to immediately begin national examination procedures (35 U.S.C. § 371(f)).

b. ☒ The U.S. National Fee (35 U.S.C. § 371(c)(1)) and other fees (37 C.F.R. § 1.492) as indicated below:

2. Fees

CLAIMS FEE	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
☐*	TOTAL CLAIMS	33 −20=	13	× $18.00=	$ 234.00
	INDEPENDENT CLAIMS	3 −3=	0	× $78.00=	
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $260.00	
BASIC FEE**	☐ U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an international preliminary examination fee as set forth in § 1.482 has been paid on the international application to the U.S. PTO: ☐ and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(1) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 C.F.R. § 1.492(a)(4)) ..... $96.00 ☐ and the above requirements are not met (37 C.F.R. § 1.492(a)(1)) ..... $670.00 ☒ U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in § 1.482 has been paid to the U.S. PTO, and payment of an international search fee as set forth in § 1.445(a)(2) to the U.S. PTO: ☐ has been paid (37 C.F.R. § 1.492(a)(2)) ..... $690.00 ☐ has not been paid (37 C.F.R. § 1.492(a)(3)) ..... $970.00 ☑ where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 C.F.R. § 1.492(a)(5)) $840.00				$840.00
	Total of above Calculations				= $1074.00
SMALL ENTITY	Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed also. (note 37 C.F.R. § 1.9, 1.27, 1.28)				− 537.00
	Subtotal				
	Total National Fee				$ 537.00
	Fee for recording the enclosed assignment document $40.00 (37 C.F.R. § 1.21(h)). (See Item 13 below). See attached "ASSIGNMENT COVER SHEET".				
TOTAL	Total Fees enclosed				$ 537.00

*See attached Preliminary Amendment Reducing the Number of Claims.

i. ☐ A check in the amount of _____ to cover the above fees is enclosed.

ii. ☒ Please charge Account No. 14-1263 in the amount of $ 537.00
A duplicate copy of this sheet is enclosed.

***WARNING:** *"To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: * * * (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 C.F.R. § 1.495(b).*

WARNING: *If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 C.F.R. § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 O.G. 29 to 40.*

3. ☒ A copy of the International application as filed (35 U.S.C. § 371(c)(2)):

NOTE: Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment. "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.

a. ☒ is transmitted herewith.

b. ☐ is not required, as the application was filed with the United States Receiving Office.

c. ☒ has been transmitted

i. ☐ by the International Bureau.
Date of mailing of the application (from form PCT/1B/308): _____.

ii. ☐ by applicant on _____.
Date

4. ☒ A translation of the International application into the English language (35 U.S.C. § 371(c)(2)):

a. ☐ is transmitted herewith.

b. ☐ is not required as the application was filed in English.

c. ☐ was previously transmitted by applicant on _____.
Date

d. ☒ will follow.

5. ☐ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. § 371(c)(3)):

NOTE: The Notice of January 7, 1993 points out that 37 C.F.R. § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.

a. ☐ are transmitted herewith.

b. ☐ have been transmitted

i. ☐ by the International Bureau.
Date of mailing of the amendment (from form PCT/1B/308): _____.

ii. ☐ by applicant on (date) _____.
Date

c. ☒ have not been transmitted as

i. ☒ applicant chose not to make amendments under PCT Article 19.
Date of mailing of Search Report (from form PCT/ISA/210.): _____.

ii. ☐ the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. ☐ A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. § 371(c)(3)):

a. ☐ is transmitted herewith.

b. ☐ is not required as the amendments were made in the English language.

c. ☐ has not been transmitted for reasons indicated at point 5(c) above.

7. ☐ A copy of the international examination report (PCT/IPEA/409)

☐ is transmitted herewith.

☐ is not required as the application was filed with the United States Receiving Office.

8. ☐ Annex(es) to the international preliminary examination report

a. ☐ is/are transmitted herewith.

b. ☐ is/are not required as the application was filed with the United States Receiving Office.

9. ☐ A translation of the annexes to the international preliminary examination report

a. ☐ is transmitted herewith.

b. ☐ is not required as the annexes are in the English language.

10. ☒ An oath or declaration of the inventor (35 U.S.C. § 371(c)(4)) complying with 35 U.S.C. § 115

a. ☐ was previously submitted by applicant on _____. Date

b. ☐ is submitted herewith, and such oath or declaration

i. ☐ is attached to the application.

ii. ☐ identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. § 1.70.

c. ☒ will follow.

II. Other document(s) or information included:

11. ☒ An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):

a. ☐ is transmitted herewith.

b. ☒ has been transmitted by the International Bureau. Date of mailing (from form PCT/IB/308): _____.

c. ☐ is not required, as the application was searched by the United States International Searching Authority.

d. ☐ will be transmitted promptly upon request.

e. ☐ has been submitted by applicant on _____. Date

12. ☐ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98:

a. ☐ is transmitted herewith.

Also transmitted herewith is/are:

☐ Form PTO-1449 (PTO/SB/08A and 08B).

☐ Copies of citations listed.

b. ☐ will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. § 371(c).

c. ☐ was previously submitted by applicant on _____. Date

13. ☐ An assignment document is transmitted herewith for recording.

A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

_____

_____

_____

14. ☒ Additional documents:

a. ☐ Copy of request (PCT/RO/101)

b. ☒ International Publication No. WO99/37761

i. ☒ Specification, claims and drawing

ii. ☐ Front page only

c. ☒ Preliminary amendment (37 C.F.R. § 1.121)

d. ☒ Other

small entity declaration

15. ☒ The above checked items are being transmitted

a. ☒ before 30 months from any claimed priority date.

b. ☐ after 30 months.

16. ☐ Certain requirements under 35 U.S.C. § 371 were previously submitted by the applicant on _____, namely:

AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: *Accurately count claims, especially multiple dependant claims, to avoid unexpected high charges if extra claims are authorized.*

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

NOTE: "Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

☒ The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 14-1262.

☒ 37 C.F.R. § 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: *Because failure to pay the national fee within 30 months without extension (37 C.F.R. § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.*

☐ 37 C.F.R. § 1.492(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.

☐ 37 C.F.R. § 1.17 (application processing fees)

☐ 37 C.F.R. § 1.17(a)(1)–(5) (extension fees pursuant to § 1.136(a).

☐ 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).

NOTE: 37 C.F.R. § 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

☐ 37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).

/SIGNATURE OF PRACTITIONER

Reg. No.: 33,531

Tel. No.: (212) 968 1300

Customer No.:

Bruce S. Londa
(type or print name of practitioner)

Norris, McLaughlin & Marcus, P.A.
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20 Exchange Place, 37th fl.
New York, New York 10005

Attn. Mr. Londa!

Applicant or Patentee: Hoehe et al.
Serial or Patent Number: PCT/DE98/03818 Attorney's Docket No
Filed or Issued:
For: Novel Sequence Variants of the Human Beta2-Adrenergic Gene

Verified Statement (Declaration) Claiming SMALL ENTITY
Status (37 CFR 1.9(f) and 1.27 (d)) - Small Business Concern

I hereby declare that I am

[ ] the owner of the small business concern identified below
[X] an official of the small business concern empowered to act on behalf of the concern identified below

NAME OF CONCERN Max-Delbrück-Centrum für Molekulare Medizin
ADDRESS OF CONCERN Robert-Rössle-Strasse 10, Berlin D-13125, Germany

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled

Novel Sequence Variants of the Human Beta2-Adrenergic Gene

by inventor(s) Hoehe et al

described in PCT/DE98/03818, filed 30 December 1998

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

*

NAME: _____
ADDRESS: _____
[ ] Individual [ ] Small Business Concern [ ] Nonprofit Organization

NAME: _____

ADDRESS: _____

[ ] Individual [ ] Small Business Concern [ ] Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: _____

TITLE OF PERSON OTHER THAN OWNER: _____

ADDRESS OF PERSON SIGNING: _____

SIGNATURE F. Baumbach DATE 29.06.00

by order!

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty's Docket No: 101195-2

Applicant(s) : Hoehe et al

Filed : Concurrently herewith

For : Novel sequence variants of the human beta2-adrenergic gene

PRELIMINARY AMENDMENT

Hon. Assistant Commissioner of Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination, please amend the application as follows:

IN THE CLAIMS

Please amend claims 3-8 to depend solely from claim 1.

Please amend claims 14, 16, 17, 18, 19, 21, 22, 23, to depend solely from claim 9.

Please amend claim 24 to depend solely from claim 1.

Please amend claims 27, 28, 29 to depend solely from claim 9.

Please amend claim 30 to depend solely from claim 1.

Please amend claims 31 and 32 to depend solely from claim 24.

Please amend claim 33 to depend solely from claim 1.

REMARKS

The above amendments were made to place the application into

proper United States Patent format. Early and favorable consideration is earnestly solicited.

Respectfully Submitted,

Bruce S. Londa (33,531)
Attorney for Applicant
Norris, McLaughlin & Marcus
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09/582719
Rec'd PCT/PTC 22 AUG 2000
PCT/DE98/03818

Novel sequence variants of the human beta2-adrenergic receptor gene and use thereof

The invention relates to novel sequence variants of the human beta2-adrenergic receptor gene and to their use for diagnosing a range of diseases, in particular for detecting dispositions to high blood pressure and for developing therapeutic agents on the basis of pharmacogenetic principles

The human beta2-adrenergic receptor is an important component of the sympathetic nervous system, regulating as such a range of central and peripheral functions such as cardiovascular functions, metabolic functions, central nervous functions and neurosecretion. It is the point of attack for pharmaceutical/therapeutic agents with a broad range of indication belonging to drugs administered most frequently. Manifold findings point to the fact that this receptor might play a part in the pathogenesis/pathophysiology of a number of frequent diseases such as e.g. hypertension and other cardiovascular diseases, various neuropsychiatric diseases such as e.g. depression and metabolic diseases such as e.g. obesity (Insel PA (Ed) (1987) Adrenergic receptors in man, Marcel Dekker, New York, Basel).
The invention aims at detecting variants, polymorphisms, mutations and resulting haplotypes in the DNA sequence of the human beta2-adrenergic receptor gene and their correlations with the dispositions to diseases. Proceeding from these correlations a method for diagnosing these dispositions to diseases, for predicting the degree of severity, the course and survival time, a system for predicting the individual responsiveness to beta2 active therapeutic agents, for developing individual specific beta2 receptor agonists and antagonists and a system for developing a new class of beta2 effective therapeutic agents and for developing test systems for the investigation of pathophysiological connections and for developing the above-mentioned therapeutic agents. To sum up it is possible to predict or develop an individually optimum therapeutic agent for each beta2 genotype. The task is solved according to the claims, the subclaims are preferential variants.

It was stated that in the 5'-regulating region of the sequence of the human beta2-adrenergic receptor gene further variants are present, apart from the 3 mutations already known in the coding region (in positions 1633, 1666 and 2078). Furthermore, there was detected that these genetic variants correlate with the disposition to various diseases, e.g. high blood pressure.

Accordingly, the object of the invention is the sequence of the human beta2-adrenergic receptor gene which is entirely or partly mutated in the positions 159, 245, 565, 934, 1120, 1221, 1541, 1568, 1633, 1666, 1839, 2078, 2110, 2640 and 2826. In particular, a sequence containing entirely or partially the mutations T->A (position 159), A->G (position 245), G->A (position 565), G->A (position 934), G-> C (position 1120), C->T (position 1221), C->T (Arg->Cys) position 1541), T->C (position 1668), A->G (Arg-> Gly) (position 1633), C->G (Gln->Glu) (position 1666), G->A (position 1839), C->T (Thr-> Ile) (position 2078), C->A (position 2110), G-> C (position 2640) and G-> A (position 2826) (Figs. 1, 2a and 2b) is concerned.

Especially important are the following sequences (haplotypes):

- sequence with the mutations 1541, 1633 A and 1666 C,
- sequence with the mutations 1541 C, 1633 G and 1666 G,
- sequence with the mutations 1541 T, 1633 G and 1666 C,
- sequence with the mutations 1541 T, 1568 T, 1633 A and 1666 C,
- sequence with the mutations 1541 C, 1568 C, 1633 G and 1666 G and
- sequence with the mutations 1541 T, 1568 T, 1633 G and 1666 C.

Furthermore, a method to determine dispositions to diseases is the object of the invention where all sequences and variants of the beta2-adrenergic receptor gene of the individual mutation up to any potential combinations of all variants (including any absolute number of variants which may be included) may be genotypified, allowing to furnish respective data on dispositions to diseases.

The method is characterized by the fact that the DNA of a proband is isolated and genotypified at least in one of the positions exchanged and subsequently compared with the reference DNA sequence. Forms where at least position 1633, at least the three positions 1541, 1633 and 1666 or the four last-mentioned positions (1541, 1568, 1633 and 1666) or the seven positions 245, 565, 934, 11541, 1568, 1633 and 1666 are genotypified.

The method may be also varied by genotypifying at least 3 of the 4 positions 1541, 1568, 1633 and 1666 and subsequently comparing them with the reference DNA sequence. Here, genotypifying of the positions 1541, 1633 and 1666 is preferred.

Genotypifying is carried out by sequencing or by means of other methods suited for the detection of point mutations. They involve PCR-aided genotypification methods such as e.g. allel-specific PCR, other genotypification methods using oligonucleotides (examples would be 'dot blotting' or 'oligonucleotide ligation assays' (OLA), methods using restriction enzymes and 'single nucleotide polymorphism ' (SNP) analysis by means of 'matrix-assisted laser desorption/ionization mass spectrometry (MALDI) and, in principle, any method to detect variants which will be available in future including chip technology in all its technological variants.

Proceeding on it, the method according to the present invention for determining a broad spectrum of most various dispositions to diseases is suited.

In a variant it is suited e.g. for the determination of high blood pressure (or for predicting the region of the individual high blood pressure values per se), and other cardiovascular diseases including myocardial infarct and apoplexy, in the widest sense the development of a terminal renal insufficiency (being in need of dialysis).

A further preferential variant allows e.g. to determine a disposition to neuropsychiatric diseases such as depressions and anxiety syndromes (anxiety disorders), attention deficit disorder (with hyperactivity), eating disorder, e.g. for anorexia nervosa and bulimia, or disorder caused by posttraumatic stress; or to diseases of the autonomic nervous system such as e.g. Bradbury-Eggelston, Sky-Drager and Riley-Day syndromes and selective noradrenergic and baroreceptor dispositions or migrain.

In addition, it is also suited for detecting dispositions to allergic diseases, in particular asthma and atopic disorder.

A further application is the determination of a disposition to metabolic diseases such as obesity (and family "morbid obesity") including a prediction of the weight area as such and a disposition to change of weight, finally a prediction of the proportion of the measurements of the body as such as they are e.g. expressed in the "body mass index" (BMI).

Furthermore, the method allows also the determination of the course and the severity of diseases and the prediction of survival after severe medical diseases, e.g. after myocardial infarct, cardiac failure and/or apoplexy.

A further preferential variant allows the determination of an individually varying reactivity of the autonomic nervous system, in particular to endogenous and exogenous stress (as it is, e. g. in particular, expressed by an individually varying disposition to high blood pressure and/or heart rate modifications (deflections) or individually differing blood pressure modifications as a result of endogenously or exogenously induced changes of the salt concentration in blood (individually varying sensitivity or resistance to salt) and, in the widest sense, also by the individually varying salt and water regulation or reverse resorption in the kidney (volume regulation connected with it).

A further important object of the invention is the use of the required sequence variants a) for predicting the individually varying responsiveness to therapeutics known so far (beta2 receptor ligands) and the individually varying responsiveness to the endogenous ligands adrenalin and noradrenalin; b) preferably for developing individually specific beta2 receptor agonists and antagonists; c) in particular, also for developing a new class of therapeutics directed to the beta2 receptor gene which attack at the 5'regulatory region, promoter region, in particular e. g. at the leader peptide, and have effect via regulation of transcription, translation or affecting its efficiency, in particular by regulating the expression.
In this connection, a further object of the invention is predicting the individual habituation to the administration of pharmaceutical agents (tachyphylaxis) and a various disposition to side effects of pharmaceutical agents. Altogether, a prediction of individually optimum therapeutics on which various effective mechanisms are based is possible

A further important object of the invention is the use of the claimed sequence variants for building up genes or vectors, in particular for the development of pharmaceutically relevant substances and for the development of a diagnostic kit or any diagnostic method. Such kits and methods may be used with a favourable effect for predicting the individual disposition to diseases or the individual responsiveness to various beta2-therapeutics.

Thus, cultures (cells) expressing the most various combinations of individual ß2-variants mentioned may serve as test models for the development of individual specific therapeutics (ß2-agonists and antagonists and beta2-expression regulating DNA therapeutics). This corresponds to test models *in vitro*, yet also *in vivo* test models are included (transgenic animals bearing these individual receptor variants).

As individual test models they allow in vitro (= ex vivo) a prediction of the individual functional state of the beta 2-receptor or the functions mediated by it.

The extent of the claimed invention is represented in detail hereinafter. To prepare the invention the whole DNA sequence known of the human beta2-adrenergic receptor gene including its regulating and coding regions in patients and checks by means of 'multiplex PCR sequencing' are investigated and, first of all, a number of genetic variants is identified. In the 5' regulating region eight new variants have so far been detected the most important of which seems to be the substitution of a highly preserved Arg->Cys in the 'leader peptide' of the gene (position 1541) which regulates the translation of the receptor gene (position –47 in relation to the starting point of translation), i. e. its expression.

Summary of the newly identified variants (nucleotide position before the substitution is related to the beta2-receptor gene sequence published (Koliba B.K. et al., Proc. Natl. Acad. Sci USA; 84(1): 46-50 (1987) [Acc No. JO2960]; the information in brackets behind the substitution refers to the start of translation).

159 T -> A (-1429)
245 A -> G (-1343)
565 G -> A (-1023)
934 G -> A –654)
1120 G -> C (-468)
1221 C -> T (-367)
1541 C -> T (-47) Arg -> Cys substitution in the 'leader peptide' of the beta2 receptor gene
1568 T -> C (-20)

These variants are clearly represented in Figs. 1, 2a and 2b.

Correlations with diseases or clinically relevant phenotypes:

Specific effects of the two mutations known so far Arg -> Gly (in position +46 related to the starting point of translation, corresponds to position 16 of the amino acid sequence) and Gln -> Glu (in position +79 related to the starting point of translation, corresponds to position 27 of the amino acid sequence) and the newly detected 'leader peptide' mutation Arg -> Cys (in position –47 related to the starting point of translation) on a number of clinically and pathogenically relevant phenotypes were detected in a few studies. Thus, a significant association of the alleles in position 16 of the amino acid sequence with the genetic predisposition to hypertonia and extremely deflected blood pressure values was detected. The three mutations described hereinafter have a significant effect on phenotypical parameters such as heart rate, noradrenalin concentrations, blood pressure modifications as a result of experimentally induced physical and mental stress, 'coping styles' and personality dimensions such as weight and change of weight. In particular, also an association of the 'leader peptide' mutation with hypertonia was shown. Furthermore, it was possible to establish a relation between beta2-agonist induced vasodilatation and beta2 receptor mutations, preferably in position 16 of the amino acid sequence, and a relation between beta2 receptor expression of individuals genotypified in fibroblast cultures and beta2 receptor mutations, preferably in position 16 of the amino acid sequence.

Detection of specific three-mutation combinations in positions (related to the beta 2-sequence published, Kobilka et al. 1987) 1541 C -> T ('leader peptide' mutation Arg -> Cys), 1633 A -> G (Arg -> Gly) and 1666 C -> G (Gln -> Glu):

combination 1 : 1541 T (Cys allel), 1633 A (Arg allel), 1666 C (Gln allel)

combination 2: 1541 C Arg allel), 1633 G (Gly allel), 1666 G (Glu allel)

combination 3: 1541 T (Cys allel), 1633 G (Gly allel, 1666 C (Gln allel)

These three specific combinations occur in 80 – 95 % of the population, they seem to be selected evolutionarily from the total number of combinations to be expected and represent various functional states of the human beta 2-adrenergic receptor on which the variability of physiological and pathophysiological functions is based In particular, they are connected with an individually varying responsiveness to endogenous ligands such as adrenalin and noradrenalin and with a various therapeutical responsiveness to beta2 receptor agnostis and

antagonists which enables these 'combinations' to be a starting point for the development of an 'individually tailored pharmacotherapy'.

Detection of specific beta2 'haplotypes' consisting of four variants: in the positions (related to the beta2 sequence published, Kobilka et al. 1987) 1541 C -> T ('leader peptide' mutation Arg -> Cys), 1568 T -> C; 1633 A -> G (Arg -> Gly) and 1666 C -> G (Gln -> Glu):

Combination 1: 1541 (Cys allel), 1568 T, 1633 A (Arg allel), 1666 C (Gln allel)
Combination 2: 1541 C (Arg allel), 1568 C, 1633 G (Gly allel), 1666 G (Glu allel)
Combination 3: 1541 T (Cys allel), 1568 T, 1633 G (Gly allel), 1666 C Gln allel)

Combination 1 was observed significantly more frequently in individuals having an inclination towards hypertonia, thus representing a genetic risk factor.

Detection of specific beta2 'haplotypes' consisting of seven variants:
Considering all variants in calculations it was possible to extract 'haplotypes' consisting of seven variants (including the three mutations mentioned); the calculations were aimed at identifying 'haplotypes' from the entirety of the genome which were sufficient to distinguish between the patient group and the control group. A specific 'haplotype', combination 1, may be more frequently observed in the case of a genetic loading by hypertonia. This may be extended to other phenotypes.

Combination 1:	245 G, 565 G, 934 A, 1541 T (Cys allel), 1568 T, 1633 A (Arg allel), 1666 C (Gln allel)
Combination 2.	245 A, 565 A, 934 G, 1541 C (Arg allel), 1568 C, 1633 G (Gly allel), 1666 G (Glu allel)
Combination 3.	245 G, 565 G, 934 G, 1541 T (Cys allel), 1568 T, 1633 G (gly allel), 1666 C (Gln allel)

The "haplotypes" described last describe finally the real, total individual functional state of the receptor. The invention is based on the concept that the various functional (dysfunctional) receptor states are not based on individual mutations but are a result of the individual "polymorphic" overall gene sequence as a function determining unity.
Subsequently, the invention is explained in greater detail by an example.

Material and methods

The multiplex PCR sequencing method is applied for ascertaining the total polymorphic spectrum of the beta2 receptor gene. To this end, the overall promoter region known so far and the coding region are subdivided into eight fragments and amplified by means of PCR (see Fig. 1). These PCR fragments were pooled and sequenced simultaneously. The fragments of the termination reactions were separated on a sequence gel and transferred to a nylon membrane by means of direct transfer electrophoresis (DTE). The individual sequence leaders were successively decoded by successively hybriziding with specific oligonucleotides.

The specific conditions for the amplification were as follows:

Forward primer ADRBR-F1 with the sequence 5'-TATTGGCCAGGATCTTTTGCTTTCTAT-3' and backward primer ADRBR-R1 with the sequence 5'-TAACATTAAGAACATTTTGAAGC-3' were used for fragment I. Fragment II was amplified by means of the two primers ADRBR-F2: 5'-GCATACCCCCGCTCCAGATAAA-3' and ADRBR-R2: 5'-GCACGCACATACAGGCACAAATAC-3'. For fragment III it were two primers ADRBR-F3: 5'-GGCCGCGTTTCTGTGTTGG-3' and ADRBR-R3: 5'-AGTGCGTTCTGCCCGTTATGTG-3'. For fragment VIII the two primers ADRBR-F8: 5'-GGTACTGTGCCTAGCGATAAC-3' and ADRBR-R8: 5'-TAAAATACCCCGTGTGAGCAAATAAGAG-3' were used. The reaction conditions for these four fragments were as follows: 10 x PCR buffer (100 mM Tris HCl, 15 mM $MgCl_2$ x 6 H_2O, 500 mM KCl, pH 8.3), dNTP 2 mM, 30µM primer F, 30 µM primer R, 50 ng of genomic DNA and 5 U of a *Taq* DNA polymerase. All three fragments were amplified with the following temperature profile: 94° C 4 min; 35 cycles: 94°C 30 sec., 60°C 30 sec., 72 °C 1 min. and finally 72°C 10 min

Fragment IV was amplified with the aid of the two primers ADRBR-F4: 5'-GGGGAGGGAAAGGGGAGGAG-3' and ADRBR-R4: 5'-CTGCCAGGCCCATGACCAGAT-3'. For fragment VII the primers ADRBR-F7: 5'-CTGGCTGCCCTTCTTCATCGTT-3' and ADRBR-R7: 5'-TACCCTAAGTTAAATAGTCTGTT-3' were used. The conditions for these two PCR reactions were as follows: 10 x PCR buffer (160 mM $(NH_4)_2SO_4$, 0.1 % of Tween-20, 500 mM KOH, pH), dNTP 2 mM, 30 µM primer F, 30 µM primer R, 50 ng of genomic DNA and 4 U of a mixture of *Taq* DNA polymerase and a thermostable inorganic pyrophosphatase of *thermus thermophilus*. Both fragments were amplified with the following temperature profile:

94°C 4 min.; 35 cycles: 94°C 30 sec., 66°C [fragment IV] or 60°C [fragment VII] 30 sec., 72°C 1 min. and finally 72°C 10 min.

Fragment V was amplified by means of the two primers ADRBR-F5: 5'-ATGCGCCGGACCACGAC-3' and ADRBR-R5: 5'-GTAGAAGGACACGATGGA-3', fragment VI was amplified with the two primers ADRBR-R6: 5'-GCTACTTTGCCATTACTTCACC-3' and ADRBR-R6: 5'-AAATCTGGGCTCCGGCAGTAGATAAG-3'. These two fragments were amplified by means of 'AmpliTaq gold kits' by Perkin Elmer. In these two fragments the temperature profile was as follows: 94°C 10 min.; 35 cycles: 94°C 30 sec., 56 °C [fragment V] or 58°C [fragment VI] 30 sec., 72 °C 1 min. and finally 72°C 10 min.

Sequencing was carried out by means of the 'thermo sequenase cycle sequencing kit' by Amersham. The PCR primers described above were used as sequencing primers. Sequencing was carried out in four multiplex pools. Pool 1 contained the sequencing primers ADRBR-F1, ADRBR-F3, ADRBR-F5 and ADRBR-F7; pool 2 contained the sequencing primers ADRBR-R1, ADRBR-R3, ADRBR-R5 and ADRBR-R7. Fragments I, III, V and VII were inserted into the two sequencing pools. Yet, pool 3 contained the sequencing primers ADRBR-F2, F4, F6 and F8; pool 4 contained the sequencing primers ADRBR-R2, R4, R6 and R8. Fragments II, IV, VI and VIII were inserted into these two pools.

All PCR and sequencing reactions were carried out in a PTC 225 cycler of MJ Research.

The products of the sequencing reaction were separated on a 100 µm thick acryl amide gel (5% acryl amide, 7 M urea) and under standard DTE conditions (see Richterich and Church, 1993) transferred to a biodyne A membrane (Pall). Then, the membrane was hybrizided with ^{32}P-marked oligonucleotides and the individual sequence leaders were detected with the aid of a phospho fluorimager (Storm 860, Molecular Dynamics).

Literature:

Kobilka, B.K., Dixon R.A., Frielle T.; Dohlman H. G., Bolanowski M.A., Sigal I.S., Yang Feng T.L., Francke U., Caron M.G., Lefkowitz R.J.: cDNA for the beta 2-adrenergic receptor: a protein with multiple membrane-spanning domains and encoded by a gene whose chromosomal location is shared with that of the receptor for platelet-derived growth factor. *Proc Natl Acad Sci USA*; 84 (1): 46-50 (1987).

Parola A. L. and Kobila B.K. The peptide product of a 5' leader cistron in the beta 2-adrenergic receptor mRNA inhibits receptor synthesis. *J Biol chem*. 269 (6): 4497-505 (1994).

Richterich P. and Church G.M.: DNA sequencing with direct transfer electrophoresis and nonradioactive detection. *Methods Enzymol*. 218: 187-222 (1993).

Legends relating to the Figures:

Fig. 1

Polymorphic spectrum of the human beta 2-adrenergic receptor gene
Variants are indicated according to their nucleotide positions.
(Reference sequence Kobilka et al. 1987).

Fig. 2a

Sequence of the human beta 2-adrenergic receptor (Kobilka et al. 1987)
Variants are indicated according to their positions.

Fig. 2b

Sequence of the human beta 2-adrenergic receptor (Kobilka et al. 1997).
The variants (nucleotide or amino acid substitution) are indicated.

Patent claims

1. Sequence of the human beta2-adrenergic receptor gene wherein the bases have been substituted completely or partly in the positions 159, 245, 565, 934, 1120, 1221, 1541, 1568, 1633, 1666, 1839, 2078, 2110, 2640 and 2826.

2. Sequence according to claim 1 wherein it involves completely or partly the substitution of bases T -> A (position 159), A -> G (position 245), G -> A (position 565), G -> A (position 934), G -> C (position 1120), C –T (position 1221), C -> T (position 1541), T -> C (position 1568), A -> G (position 1633), C -> G (position 1666), G -> A, (position 1839), C -> T (position 2078), C -> A (position 2110), G -> C (position 2640) and G -> A (position 2826).

3. Sequence according to claims 1 and 2 characterized by the mutations 1541 T, 1633 A and 1666 C.

4. Sequence according to claims 1 and 2 characterized by the mutations 1541 C, 1633 G and 1666 G.

5. Sequence according to claims 1 and 2 characterized by the mutations 1541 T, 1633 G and 1666 C.

6. Sequence according to claims 1 and 2 characterized by the mutations 1541 T, 1568 T, 1633 A and 1666 C.

7. Sequence according to claims 1 and 2 characterized by the mutations 1541 C, 1568 C, 1633 G and 1666 G.

8. Sequence according to claims 1 and 2 characterized by the mutations 1541 T, 1568 T, 1633 G and 1666 C.

9. Method for determining dispositions to diseases wherein the DNA of a proband is extracted and genotypified at least in one of the substituted positions and subsequently compared with the reference DNA sequence, if necessary, with all potential

combinations of variants from the individual mutation to all potential combinations of all variants being included, including any absolute number of variants.

10. Method according to claim 9 wherein the DNA of a proband is extracted and genotypified at least in position 1633.

11. Method according to claim 9 wherein the DNA of a proband is extracted and genotypified at least in the three positions 1541, 1633 and 1666.

12. Method according to claim 9 wherein the DNA of a proband is extracted and genotypified at least in the four positions 1541, 1568, 1633 and 1666.

13. Method according to claim 9 wherein the DNA of a proband is extracted and genotypified at least in the seven positions 245, 565, 934, 1541, 1568, 1633 and 1666.

14. Method according to claims 9 or 12 wherein the positions 1541, 1568, 1633 and 1666 are genotypified.

15. Method according to claim 14 wherein at least 3 of the 4 positions 1541, 1568, 1633 and 1666 are genotypified.

16. Method for determining the dispositions to diseases according to the claims 9, 11 or 15 wherein the positions 1541, 1633 and 1666 are genotypified.

17. Method according to one of the claims 9 to 16 wherein genotypifying is brought about by sequencing or other methods suited for detecting variants.

18. Methods according to one of the claims 9 to 17 for determining a disposition to high blood pressure and deviations of the blood pressure from the standard and other cardiovascular diseases including myocardial infarct and apoplexy; for determining a disposition to neuropsychiatric diseases such as depression, anxiety syndromes, attention deficit disorder with hyperactivity, eating disorder, e.g. anorexia nervosa and bulimia or disorders caused by post-traumatic stress; for determining a disposition to

diseases of the autonomic nervous system such as e.g. Bradbury-Eggleston, Sky-Drager and Riley-Day syndromes and dispositions to selective noradrenergic and baroreceptors or migrain; for determining a disposition to allergic diseases, in particular asthma and atopic disorder; for determining a disposition to metabolic diseases such as obesity and family "morbid obesity", including a prediction of the weight area as such or a disposition to a change of weight, including a prediction of the proportion of the measurements of the body as such as expressed e.g. in the "body mass index" (BMI).

19. Method according to one of the claims 9 to 17 for determining an individually different reactivity of the autonomic nervous system, in particular to endogenous and exogenous stress.

20. Method according to claim 19 for determining an individually different disposition to modification/deflections of blood pressure and/or heart rate caused by endogenous and exogenous stress or an individually different sensitivity/resistance to salt

21. Method according to one of the claims 9 to 17 for determining the course and the degree of severity of diseases such as e.g. mentioned in claim 18, e.g. of neuropsychiatric diseases such as depression and anxiety syndromes, of cardiovascular diseases including myocardial infarct and apoplexy, of diseases of the autonomic nervous system and allergic diseases such as e. g. asthma.

22. Method according to one of the claims 9 to 17 for determining a disposition to metabolic diseases such as obesity.

23. Methods according to one of the claims 9 to 17 for predicting the survival time after severe diseases such as after a myocardial infarct, cardiac failure and/or apoplexy.

24. Use of sequence variants according to claims 1 to 8 for developing therapeutic agents and/or lifestyle drugs.

25. Use according to claim 24 for developing a new class of therapeutic agents directed to the beta2 receptor gene and attacking the 5'regulatory area, the promoter area and the

leader peptide, active via the regulation of transcription and translation, and by affecting their efficiency, notably by regulating the expression.

26. Use according to claim 24 for developing beta2 receptor agonists and antagonists, in particular individually specific beta2 receptor agonists and antagonists.

27 Use according to one of the claims 9 to 17 for predicting the individually different responsiveness to so far known therapeutic agents such as beta2 receptor ligands and therapeutic agents developed in future also under claims 24 to 26 and the individually different responsiveness to the endogenous ligands adrenalin and noradrenalin.

28 Use according to one of the claims 9 to 17 for predicting the individual habituation to the administration of pharmaceutical agents (tachyphylaxis) and a different disposition to side effects of pharmaceutical agents.

29. Use according to one of the claims 9 to 17 to optimize the individual therapy or intervention directed to the beta2 receptor and its gene.

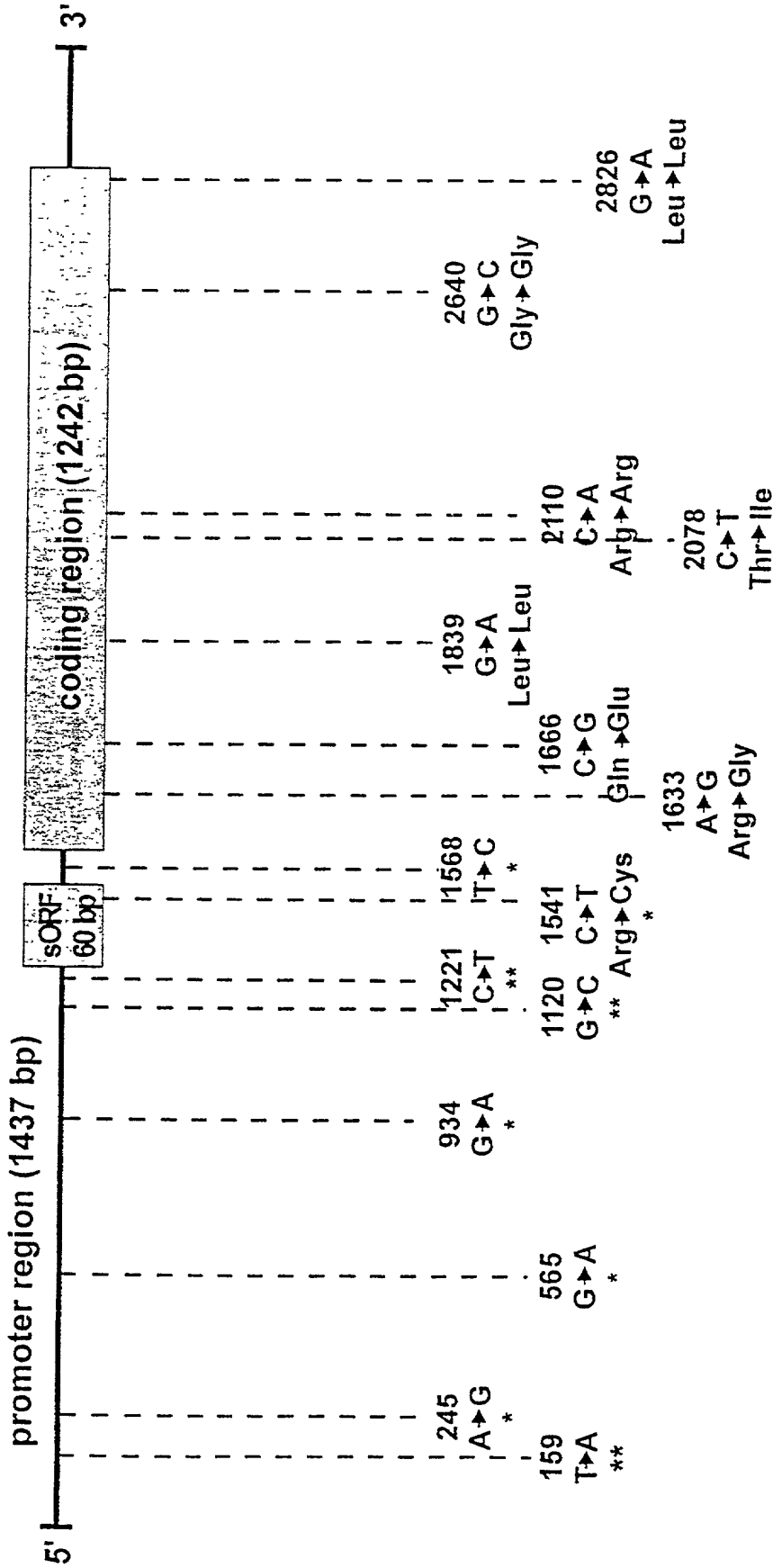
30. Use of sequence variants according to claims 1 to 8 to build up genes or vectors, in particular to develop pharmaceutically relevant substances

31. Use according to claims 24 to 30 for developing a diagnostic kit or an optional method for genotypifying.

32. Use according to claims 24 to 31 for developing a diagnostic kit for predicting the individual responsiveness to various beta2 receptor agonists and antagonists and to any newly developed beta2 active therapeutic agents, in particular also according to claim 25; for predicting the therapeutic efficiency of pharmaceutical agents the action mechanism of which involves modifications of the beta2 receptor structure, regulation or expression; for predicting the individually different responsiveness to the endogenous ligands adrenalin and noradrenalin; for predicting the individual habituation to pharmaceutical agents administered – tachyphylaxis – and a different disposition to side

effects of pharmaceutical agents; for optimizing the individual therapy or intervention to the beta2 receptor and its gene.

33. Use according to claims 1 to 8 and 9 to 32 for developing in vitro (e.g. cell cultures) and in vivo (e.g. transgenic animals) test systems expressing individual forms of the beta2 receptor gene, with the test systems serving to investigate the pathophysiology of diseases of in general medically important properties, with the beta2 receptor gene participating, and for developing and testing individually specific therapeutic agents agents and "lifestyle drugs" and substances directed to beta2 in general.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promoter region (1437 bp)
sORF
60 bp
coding region (1242 bp)
3'
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565 G→A *
934 G→A *
1120 G→C **
1221 C→T **
1541 C→T Arg→Cys *
1568 T→C *
1633 A→G Arg→Gly
1666 C→G Gln→Glu
1839 G→A Leu→Leu
2078 C→T Thr→Ile
2110 C→A Arg→Arg
2640 G→C Gly→Gly
2826 G→A Leu→Leu

Abb. 1

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2761 ggtactgtgc ctagcgataa cattgattca caagggagga attgtagtac aaatgactca

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Abb. 2a

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1741 gtgctggtca tcacagccat tgccaagttc gagcgtctgc agacggtcac caactacttc
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g (Arg 16 → Gly)

t (Arg → Cys)

g (Gln 27 → Glu)

a (Leu 84 → Leu)

1801 atcacttcac tggcctgtgc tgatctggtc atgggcctgg cagtggtgcc ctttggggcc

1861 gcccatattc ttatgaaaat gtggactttt ggcaacttct ggtgcgagtt ttggacttcc

1921 attgatgtgc tgtgcgtcac ggccagcatt gagaccctgt gcgtgatcgc agtggatcgc

1981 tactttgcca ttacttcacc tttcaagtac cagagcctgc tgaccaagaa taaggcccgg

t (Thr 164 → Ile)

2041 gtgatcattc tgatggtgtg gattgtgtca ggccttacct ccttcttgcc cattcagatg

a (Arg 175 → Arg)

2101 cactggtacc gggccaccca ccaggaagcc atcaactgct atgccaatga gacctgctgt

2161 gacttcttca cgaaccaagc ctatgccatt gcctcttcca tcgtgtcctt ctacgttccc

2221 ctggtgatca tggtcttcgt ctactccagg gtctttcagg aggccaaaag gcagctccag

2281 aagattgaca aatctgaggg ccgcttccat gtccagaacc ttagccaggt ggagcaggat

2341 gggcggacgg ggcatggact ccgcagatct tccaagttct gcttgaagga gcacaaagcc

2401 ctcaagacgt taggcatcat catgggcact ttcaccctct gctggctgcc cttcttcatc

2461 gttaacattg tgcatgtgat ccaggataac ctcatccgta aggaagttta catcctccta

2521 aattggatag gctatgtcaa ttctggtttc aatcccctta tctactgccg gagcccagat

(Gly351→Gly) c

2581 ttcaggattg ccttccagga gcttctgtgc ctgcgcaggt cttctttgaa ggcctatggg

2641 aatggctact ccagcaacgg caacacaggg gagcagagtg gatatcacgt ggaacaggag

2701 aaagaaaata aactgctgtg tgaagacctc ccaggcacgg aagactttgt gggccatcaa

2761 ggtactgtgc ctagcgataa cattgattca caagggagga attgtagtac aaatgactca

a (Leu 413 → Leu)

2821 ctgctgtaaa gcagtttttc tacttttaaa gacccccccc cccccaacag aacactaaac

2881 agactattta acttgagggt aataaactta gaataaaatt gtaaaaattg tatagagata

2941 tgcagaagga agggcatcct tctgcctttt ttatttttt aagctgtaaa aagagagaaa

3001 acttatttga gtgattattt gttatttgta cagttcagtt cctctttgca tggaatttgt

3061 aagtttatgt ctaaagagct ttagtcctag aggacctgag tctgctatat tttcatgact

3121 tttccatgta tctacctcac tattcaagta ttaggggtaa tatattgctg ctggtaattt

3181 gtatctgaag gagattttcc ttcctacacc cttggacttg aggattttga gtatctcgga

3241 cctttcagct gtgaacatgg actcttcccc cactcctctt atttgctcac acggggtatt

3301 ttaggcaggg atttgaggag cagcttcagt tgttttcccg agcaaaggtc taaagtttac

3361 agtaaataaa atgtttgacc atgccttcat tgcacctgtt tgtccaaaac cccttgactg

3421 gagtgctgtt gcctcccccа ctggaaaccg c

Abb. 26

If each inventor understands English, the Declaration and Power of Attorney below is suitable for use when filing a regular patent application and also when entering the national stage, in the case of an International application designating the USA under the PCT.

NORRIS, MCLAUGHLIN & MARCUS, P.A.
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COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION	Attorney Docket No.

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name,
I believe I am the original, first and sole inventor (if only one name is listed below at 201) or an original, first and joint inventor (if plural names are listed below at 201-206) of the subject matter which is claimed and for which a patent is sought on the invention entitled

the specification of which (check one)

____ is attached hereto

____ was filed on _____

____ under Serial Number _____ and was amended on _____(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56.

I list below any prior foreign application(s) for patent or inventor's certificate in respect of which foreign priority benefits are claimed under 35 USC 119; and any prior foreign application(s) for patent or inventor's certificate in respect of which such foreign priority rights are not claimed and which has a filing date before that of any application in respect of which such foreign priority benefits are claimed:

Application Number	Country	Filing Date (day, month, year)	Priority Claimed under 35 USC 119
			YES:___ NO:___
			YES:___ NO:___
			YES:___ NO:___

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

Application No.	Filing Date

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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206	Family Name	First Given Name	Second Give n Name
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under secton 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 201	Date 27.7.2000
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Signature of Inventor 203	Date 27-7-2000
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